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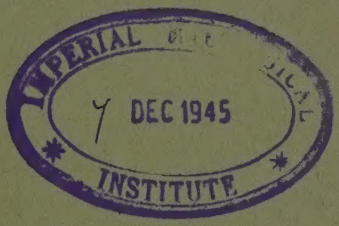

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THE BOTANICAL REVIEW

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HETEROSIS

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INTRODUCTION

Hybrid vigor, the manifest superiority of certain hybrids in size, yield and general vegetative vigor, has been recognized for at least two and a half centuries. The first artificially produced plant hybrids studied, those originating from crosses made by Kölreuter (80), furnished some excellent examples. Knight, the English horticulturist (79), noted the superiority of hybrids over pure types in many plants, and concluded that "nature intended that a sexual intercourse should take place between neighboring plants of the same species"—a conclusion which was to be elaborated in another half century by Darwin. Other very extensive work points to early recognition of wide-spread occurrence of the phenomenon (48, 52). Mendel's famous paper (95) contains a statement reporting hybrids of six to seven and one-half feet in height from crosses between parents with heights of one foot and six feet, respectively. Jones (67) cites this case as an example of hybrid vigor, but the conclusion may not be justified. Recent work tends to indicate that height is a poor measure of hybrid vigor in some plants. Darwin (27, 28) contributed many data to the problem. Paralleling the publication of Darwin's work, Beal (7) outlined a series of breeding experiments in maize to make use of the superiority of crossed strains. The American Indians had, without modern scientific knowledge, regularly planted mixtures of corn to increase the yield (23).

The observations of early investigators have been excellently summarized by Jones (67), while Collins (23) has discussed, in particular, the first work with maize. Reports prior to 1900 dealt mainly with the occurrence of hybrid vigor, and there were few suggestions

as to its causation. As Jones points out, it is to Darwin that credit must go for showing that hybrid vigor results from the union of different germinal complexes rather than from the mere act of crossing. Darwin approached the problem as no previous investigator had, through study of the effects of inbreeding. Since he was limited by working before the rediscovery of Mendel's work, he came to certain conclusions regarding the effects of inbreeding which we now know to be incorrect. Moreover, without knowledge of the Mendelian laws he was unable to recognize that inbreeding and cross-breeding are concerned with the same mechanism. Despite this limitation he saw clearly that hybrid vigor is the result of uniting organisms of differing constitutions.

THE RELATION OF HETEROZYGOSITY TO HYBRID VIGOR

It remained for G. H. Shull (117) to discern that the decrease in size accompanying inbreeding, and the increase sometimes obtained through hybridization are really different aspects of the same phenomenon. East (37) reached this same conclusion (122). In later papers (38, 41, 118, 119, 120, 121) these investigators were able to show clearly that what inbreeding does is to segregate into homozygous lines the complex aggregation of genes found in normally cross-pollinating species. They demonstrated that inbred lines capable of surviving will eventually become homozygous for all factors concerned. (The establishment of balanced lethals in certain instances presents exceptions.) When two such homozygous lines are crossed, the resulting hybrid commonly shows a marked increase in size and vigor, not only over its immediate inbred parents, but sometimes even over its more remote out-pollinating ancestors. East and Hayes (41) and G. H. Shull (121) concluded that the stimulus to development possessed by the hybrid results directly from its heterozygosity. East and Hayes stated further that up to a limiting point, which they did not define, the degree of stimulation varies directly with the number of heterozygous factor pairs. In 1910 Bruce (13) had published a short paper proposing to prove a positive correlation between dominance and vigor. In the same year Keeble and Pellew (76) published an analysis of the inheritance of stature in peas. They concluded that the added height of the F_1 was simply the result of a combination of dominant factors for larger size. Height increases were one of the most often observed manifestations of hybrid vigor. Hence,

might not hybrid vigor be explained on a similar basis? Here in the simplest terms are the two ideas around which most of the hypotheses dealing with the causal mechanism of hybrid vigor have been constructed—something inherent in the heterozygous condition on one hand, favorable action of dominant genes on the other. A third supposition, less easily definable in genetic terms, that hybrid vigor has its origin in some physiological stimulation, had been propounded before (72) and received some support about this time from A. F. Shull (116). It was at this juncture that G. H. Shull (121) proposed the term "heterosis" as a substitute for the awkward "stimulus of heterozygosity" and similar terms then in use. Shull went to considerable length to point out that more than direct gene action might be involved. He held that most of the stimulation is the result of gene action, but supposed that some of it could be derived from interaction between the male nucleus and the egg cytoplasm. Rather erroneously this term, "heterosis", has become established in the literature as a synonym for hybrid vigor. By the original definition "heterosis" refers to the developmental stimulation resulting, by whatever mechanism, from the union of different gametes. "Hybrid vigor" denotes the manifest effects of heterosis.

Before considering in detail the various proposed explanations of heterosis, it should be noted that the phenomenon is not at all confined to plants, although in them it is more easily studied and of greater realized importance. Certain early studied animal hybrids (10, 18, 53) are, however, clearly defined. Gerschler reported a cross between *Xiphophorus strigatus* (males 43.0 cm. long, females 52.0 cm.) and *Platypoecilius maculatus* (males 26.0 cm. and females 31.0 cm.) as giving "gigantic hybrids" (males 54.0 cm. and females 57.5 cm.). Bonhote obtained considerable size and fertility increases in *Meriones* crosses. Castle and Wright crossed domesticated and wild guinea pigs, obtaining hybrids whose body weights were considerably greater than those of either parent. Jones (67) has so adequately reviewed this early literature on hybrid vigor in both plants and animals that no further discussion is needed here.

THE "DOMINANCE OF LINKED GENES HYPOTHESIS"

Jones (66, 67) made what still stands as the most thorough study of the genetic aspects of the heterosis problem. He put forth a

genetic interpretation based upon the presence of certain dominant linked genes. For the basis of his hypothesis Jones returned to the earlier work of Keeble and Pellew (76) noted above. These investigators had crossed two varieties of garden peas, each of which averaged five to six feet in height. The F_1 plants averaged seven to eight feet. The F_2 fell into four classes, closely approximating a 9:3:3:1 ratio. On the basis of this segregation, the writers assumed the operation of two genes, one producing thick stems, the other long internodes. Designating these factors as T and L, respectively, they assumed one parent to be of the constitution TT-ll and the other tt-LL. Both T and L were dominant to their allelomorphs. The F_1 was, therefore, taller than either parent because it contained both factors. Subsequent investigations have cast some doubt on both the validity of these data as explaining the genetic mechanism of height in peas, and on the wisdom of calling this case one of hybrid vigor. These facts, however, do not invalidate the very good example of the manner in which dominance may cause F_1 superiority over parental types. General acceptance of this dominance hypothesis as an explanation of heterosis had been precluded by two important objections. The first objection (41, 120) is that if hybrid vigor is due to the presence of a number of dominant genes having a favorable effect on development, certain individuals in the F_2 should be found to be as good as the F_1 because by the law of segregation they would be homozygous for the favorable dominant factors. Fixation of a pure breeding superior type would thus be possible. Actually F_2 segregates do not often even closely approach the F_1 in vigor. The second objection is that (43) if dominant genes are concerned in producing the favorable effect, no matter what their number is the F_2 should show an unsymmetrical distribution for the characters involved.

By the time Jones approached the problem, however, considerably more was known about the mechanism of heredity. Most important for the explanation of heterosis was Morgan's discovery of linkage (96). Jones seized the opportunity which linkage presented for explaining away the two objections to the dominance hypothesis. He developed his theory by supposing, as is now known to be true, that large numbers of genes are concerned with characters such as height, weight and size, which operate to produce hybrid vigor. Since there are large numbers of genes concerned, it probably fol-

lows that they will be located in several different chromosomes. The occurrence of many abnormal and deficient genetically-determined characters attests that not all genes are favorable to development. As Jones pointed out, it is safe to assume that no one variety or line has all the unfavorable factors nor has any single line all the favorable ones. Since most varieties of a given species fall within the same general range of size and degree of development, it is probable that each contains the same general proportion of favorable and unfavorable genes. If a majority of the more favorable genes exhibit some degree of dominance, even though it be small, then an explanation of the superiority of the F_1 is possible. The F_1 is the only generation whose individuals will possess the maximum number of different factors. Linkage makes it impossible for any individual of the F_2 to possess as many favorable factors as the F_1, and it will also explain the absence of the skewness that might otherwise be expected.

Actually both the absence of skewness and failure to recover an equally superior F_2 individual are explainable without the assumption of linkage. Collins (24) pointed out that with any large number of factors skewness would not show up in an ordinary sized population. Singleton (122) has calculated that two thousand times the total land area of the earth would be necessary to grow enough corn plants to stand an even chance of recovering a completely homozygous plant if 30 genes were concerned.

Jones' explanation has come to be called the "Dominance of Linked Factors Hypothesis". As East (40) said, it "was so probable that it was generally accepted, at least until 1930 (2), in spite of the fact that there was no direct proof for it". During the period from 1917 to 1930 there were many publications on the subject, but most of them merely record occurrences of the phenomenon.

THE PHYSIOLOGICAL MECHANISMS—SIZE AND RATE FACTORS

In 1930 Ashby (2) began publication of a series of papers on studies of the inheritance of physiological characters. In the first paper he set forth his purpose as being that of studying, by physiological investigations, the nature of the immediate cause of hybrid vigor. Using maize he was able to determine that for the period studied (20–70 days after germination) the hybrid had the same relative growth rate as its more vigorous parent. Further, it did

not differ from either of its parents as to cell size, photosynthetic efficiency of the leaves, or time of flattening of its growth curve. The only hybrid advantages observed were higher percentage of germination and greater initial embryo weight. Ashby concluded that in this case at least the immediate cause of hybrid vigor was the larger hybrid embryo, since, as the hybrid growth rate was identical with that of the faster growing parent, this initial advantage was maintained throughout the grand period of growth. Murdoch (97) was later to demonstrate definite embryo size advantage in certain other maize hybrids. In a second set of experiments reciprocal hybrids of inbred maize lines were studied by Ashby (3). The results were essentially similar to those reported in the first paper. In each instance the hybrid appeared to have inherited the growth rate of the better parent as a simple Mendelian dominant. There was, however, considerable difference in the degree of vigor exhibited by the reciprocals. Ashby pointed out that this resulted from differences in embryo weight. The reciprocal seed weights differed, for although both were of the same genetic constitution, they had been matured on different maternal plants. The fact that embyro size is dependent upon other factors than genetic constitution may, he concluded, also explain some of the capriciousness of hybrid vigor. Ashby noted that Jones' hypothesis, based upon the action of favorable dominant genes, supposed superior metabolic efficiency on the part of the hybrids. Since his maize hybrids did not show any superiority over the parental lines by his measurements of growth rates, he came to the conclusion that some modification of the hypothesis was necessary. In the third paper (5), published in 1937, this subject is pursued further. Perhaps it is advisable to consider first some intervening papers.

Lindstrom (84) published the results of what he designated as a test of the Ashby hypothesis—the idea that hybrid vigor is due not to a greater efficiency index (relative growth rate) of the hybrid but merely to the maintenance of an initial size advantage. The test consisted of experimentally decapitating vigorous F_1 maize seedlings in early growth. Despite thus being handicapped the hybrids finally exceeded the parental strains as to dry weight of both plant and ear. This procedure did not, as Lindstrom supposed, reduce the embryonic capital in the sense in which Ashby intended the term (4). Ashby originally used the term to refer to the embryo itself,

although later he restricted its use to the meristems within the embryo. Nevertheless, it did demonstrate effectively that Lindstrom's hybrids were capable of much greater growth per unit area of leaf than were the parental types. The discrepancies between Lindstrom's results and those of Ashby probably lie, as Lindstrom suggested, in the fact that he was dealing with the entire growth period, while Ashby was considering only a restricted portion of it.

In 1936 East (40) published a detailed critique of Ashby's work and made some further observations upon the heterosis problem. Taking first the matter of seed or embryo size, East pointed out that there are several recorded cases where hybrid seeds were not as large as those of the larger parent (39, 92). Certain of these cases are in the Leguminosae where there is virtually no endosperm, hence it would appear that embryo size alone can not be an important factor. East also adduced some corollary evidence from polyploidy. In *Triticum,* particularly, there are many tetraploids and hexaploids which show greatly increased vigor, although their seeds have much reduced endosperm and embryos no larger than those of their parents. Further, he called attention to a very pertinent fact in Jones' (67) data. Jones recorded seed weights, height at given time intervals, and final yields for maize crosses. His data indicate greater growth by the F_1's than by the parents, partly attributable to their advantage in seed size. The composite curve for the F_2's shows, however, that while these plants made their start from seeds larger than those from which the F_1's grew, they actually, in the final analysis, were smaller plants. If, as Ashby supposed, relative growth rate were controlled by a single gene, with the dominant gene producing the more rapid rate, such a condition would not be possible.

The work of some other investigators is of importance in consideration of this thesis. Miss Passmore (101) noted that in reciprocal crosses in the Cucurbitaceae the cross with the larger seed reached its mature size first, but that ultimately the smaller-seeded cross equalled it. She thus disclosed the importance of a point which Ashby did not consider—duration of the growth period. Collins (106) found much the same situation in maize. The large hybrid seeds showed a significantly higher growth rate for about two weeks and then no difference was detectable. From these data and others of his own East concluded: "(1) that seed size is a nutri-

tional advantage, other things being equal; and (2) that it may be one of the manifestations of heterosis or may be the result of other causes, but that seed size, or the size of any part of the seed, can not be the true cause of heterosis." On the basis of these observations he took Ashby to task rather severely for proposing what he called the "Physiological theory of heterosis". This attitude was somewhat unfortunate, for, while at least one part of Ashby's results needed quite some qualification, Ashby recognized that he had succeeded only in pushing the problem back to the period between fertilization and maturation of the seed. He was concerned only with the immediate cause of hybrid vigor, and his evidence seemed to him indicative that, in his material, embryo size played an important part. The whole matter of embryo size in tomato has been considered in detail by Hatcher (60, 61) in papers which are a continuation of the Ashby series. Hatcher found that embryo size is dependent upon several factors, among them, truss position on the plant, number of fruit per truss, and most important, the number of fertilized ovules per fruit. He noted further that artificial pollination is much less effective than natural pollination. Since with artificial pollination fewer seeds are set, the hybrid seeds tend to be larger. He concluded that embryo size is of little importance in relation to heterosis.

Ashby's finding that the relative growth rate in his plants seemed to be determined by a single gene demands critical analysis. As Lindstrom (84) has pointed out, the existence of such a condition would be hard to understand. Ashby dealt only with a small part of the growth curve, the so-called "grand period of growth". This portion of the curve is generally supposed to represent the period of cell expansion, a supposition which may not always be entirely correct. It seems logical to assume that, other things being equal, the probability that certain environmental factors might act as "limiting factors" would be great during this period. In maize (141), at least, such seems to be the case. Inbred lines of the varieties Pipe and Pawnee and their reciprocal hybrids show markedly different reactions to varying light conditions. With some light intensities the hybrids show no superiority at all. Malinowski (92) noted that heterosis in beans produces responses similar to those obtained in certain environments. Such a condition would seem to suggest that in some crosses the hybrid will react differently than the parents to the limiting factor. Factors other than light are

also known to be concerned. Rosenquist (113) found certain wheat hybrids to be very superior when spaced four inches apart but intermediate to the parental types when spaced at one-inch intervals. Until further evidence is produced it would seem better to assume that in certain crosses the hybrids inherit a single gene, or a complex of genes, which controls their reactions to some limiting factor or group of factors. Thus the hybrids might be expected to show the same general relative growth rate as one parent at least throughout part of the growth cycle without it being necessary to suppose so complex a character as the growth rate to be determined by a single gene. Further, it is doubtful that Ashby was justified in making statements concerning growth rate without having examined the early part of the curve. As will appear later many changes can and do take place in the growth rate during and immediately after seed germination.

The 1937 paper in Ashby's series (5) deals with hybrid vigor in the tomato. In this work Ashby considered the period from germination to the onset of flowering. His growth curves are thus somewhat more inclusive, although later work (139) has shown that his initial sampling interval of 10 days was too long. The results are essentially the same as previously reported. The hybrids showed vigor in having greater dry and wet weights, more leaves each of greater area, and a greater total assimilation rate (mg. CO_2 per dm^2 per day). Despite these advantages no hybrid superiority was detected as to relative growth rate measured as dry weight or height increase, rate of production of new nodes or leaves and expansion of total leaf area, cell size (upper epidermis of first and second leaves and hypocotyl), or photosynthetic rate (increase in dry weight per unit area). From these facts Ashby deduced that: (*a*) greater dry weight of the hybrids is due to maintenance of an initial advantage in embryo weight; (*b*) greater height is attributable partly to greater length of the primordium in the embryo and possibly partly to early more rapid elongation; (*c*) greater leaf number is due to the fact that the hybrid leaves begin to unfold earlier than those of the parents; (*d*) greater leaf area results from the fact that there are more hybrid leaves, each in a later stage of development than those of the parents; and (*e*) the greater total assimilation stems from possession of greater leaf area by the hybrid.

The most significant development here is that the original capital has been redefined as consisting of the primordia within the embryo rather than the embryo as a whole. This idea was developed further and the whole study extended in the fourth paper in the series (88). Luckwill's data are concerned mostly with the flowering period. They are in strict agreement with Ashby's previous findings. He does, however, give some figures purporting to prove that the shoot primordia of the hybrids were larger than those of the parents. Unfortunately the extent of these primordia is ill-defined and the figures seem to deal with promeristem, differentiated leaves and stem tissues as a unit. Luckwill himself admitted that they show but little, and it is to be doubted that they are valid even for purposes of comparison. Luckwill pointed out again that the findings of Ashby and himself necessitate some modification of the Jones' hypothesis of dominant linked genes. From their data it appeared necessary to assume that only one or at most only a few genes are concerned in the determination of relative growth rate (post germination rate). He then suggested two alternative mechanisms for production of heterosis in the hybrid embryos. If the genes which control pre-germination growth rate are the same as those which control post-germination growth, then embryo heterosis could be attributed only to a longer embryo growth period. This longer growth period might result from some initial stimulation—the old physiological stimulation theory of hybrid vigor. If the genes controlling growth rates are not the same, then some such mechanism as Jones proposed may be operative, but only in the pre-germination stages. Even had the observations of Ashby and Luckwill been complete enough to justify their conclusions, such a distinction as that outlined above would seem both unnecessary and improbable. Little enough is known about the developmental action of genes but the idea of independent gene action appears fallacious. It is not at all likely that the physiological processes of the post-germination period are entirely independent from and unconditioned by the physiological processes of the pre-germination period. Gene action is nothing akin to the static procedure of laying one unit upon another, but is a complex, highly integrated process in which each component is in some way related to every other one. One can accept Luckwill's first proposition and the "stimulus" hypothesis, but there is no evidence for it which can not be explained better on a

genetic basis. But if one concedes that a mechanism such as that proposed by Jones is operative during development of the embryo, it would seem doubtful that dormancy, which does not even exist in some plants, would be a signal for surcease of this growth-controlling mechanism and inauguration of another. Even if this were so, the second would presumably be conditioned by the first in more than just embryo mass. As noted above, the answer seems to lie in Ashby's material in the presence of some single limiting factor operating in post-germination stages.

MANIFESTATIONS OF HETEROSIS

Heterosis is a real "double attraction". First, practical utilization of hybrid vigor is of immense value to agriculture. It has already increased the value of our corn crop by millions of dollars annually and its potentialities for other crops have hardly begun to be realized. Second, it presents ideal opportunities for the study of certain features of developmental genetics. Recent papers fall rather generally into two classes. One group has to do principally with the immediate causes and manifestations of hybrid vigor, the other with its genetic interpretation. It may be more profitable to discuss the former group first.

The prime requisite in any investigation is generally supposed to be an adequate definition of the phenomenon concerned. No such definition of hybrid vigor appears to exist. Kölreuter (80) spoke of greater size, increased number of flowers, and general vegetative vigor.

As Coffman (20) has pointed out, the effects of heterosis vary in different plants. Knight (79) noted increased height in pea crosses. Gärtner (52) recorded general vegetative luxuriance, increase in roots, height, number of flowers, and earlier and more extended period of bloom. With the advent of modern breeding methods more specific effects were recorded. For maize these are summed up by Jones (67). Among increases recorded are total yield, height, length of ear, number of nodes per plant, and number of grains per row. Root length, diameter and penetration are also greater in maize F_1's (78). Jones pointed out that the number of parts is affected much less than size of the parts. Investigators working with other plants, however, have recorded considerable increase in the number of parts, attended by little change in size. This

is particularly true in tomato (88, 89, 139). The explanation of the differences necessitates recognition of the important fact that heterosis must be expected to exhibit different manifestations in plants of unlike growth forms. East (40) gave the best idea of the way in which heterosis is manifest when he said that invariably it is something which affects the organism as a whole. "Its effect is comparable to the effect on a plant of the addition of a balanced fertilizer to the soil, or to feeding a more adequate and more chemically complete diet to the animal". Maize is a plant with a determinate growth habit. Its single shoot meristem forms a limited number of leaves and then terminates as a floral inflorescence. The number of ears per plant is limited and usually constant within a strain. While it is true that improving the growing conditions of such a plant may lengthen the vegetative period and thus bring about some increase in the number of parts, such as the number of tillers, such action is somewhat limited. Added vigor goes then into increasing the magnitude of the parts already present. Within structural limits this increase is carried on from one part of the growth cycle to another. Larger leaves make for more photosynthesis, and larger root systems for more absorption. All these factors together make for greater yield—increased mainly in maize by larger kernels on larger ears.

In tomato the situation is different. Tomato is essentially an indeterminate plant. Whaley (139) has shown that heterosis here affects the size of the parts but little. Yield is increased by greater numbers of fruit rather than by larger individual fruit. In this typical "open" growth system added vigor goes into production of new organs, and the so-called "conservative" organs, the leaves, flowers and fruits, are little affected as to size. The same situation appears to exist in the soybean. Veatch (132) reported hybrid vigor in *Soja* as increasing node number, pod number, and seed number, but having relatively little effect on size.

The ultimate manifestations of heterosis appear, then, to differ somewhat with the nature of the plant. When individual cases are considered, with growth habits of the plants concerned in mind, it is apparent that the end effects are exactly what we should expect if the immediate cause of hybrid vigor were a raising of the efficiency of metabolism. They are the sort of results derived from a superior developmental system. Once superiority has been established its

apparent degree may be considerably heightened, as G. H. Shull (121) has pointed out, by the fact that vigorous plants may be much less susceptible to the effects of varying environmental conditions.

It should be noted that investigators have used different bases for their estimations of superiority. The arithmetic mean or the geometric mean between the two parents for specific characters have been used in some instances. In other cases the hybrid was not considered to show added vigor unless it exceeded the better parent. Superiority over the better parent may be the more valid measure (21, 22, 132).

Whaley (139) made extensive experiments with hybrid vigor in tomatoes in an attempt to discover in detail some of the advantages possessed by the hybrids. Using, among others, one of the same crosses studied by Ashby and Luckwill, he found, as noted above, that heterosis produced its effects on total plant size. The determinate organs were subject to number rather than size increases. The findings supported those of Ashby as to relative growth rates during the grand period of growth, but marked differences were observed during the early post-embryonic stages and during the fruiting period. Hybrid embryo size advantage was found in one cross, but in another, showing even more hybrid vigor, the hybrid embryos were intermediate in size between those of the parents. In an attempt to test the validity of Ashby's hypothesis relating to the importance of the amount of "original meristematic capital", direct measurements were made of apical meristem volumes. Neither in the embryo nor during development was any relation found between volume of the meristem and development of hybrid vigor. In all the strains meristem volume was correlated with size of the determinate organs upon which heterosis had little if any effect. Bindloss (8) made similar observations on the size of maize meristems. However, it probably is to be expected that meristem size in the embryo will be conditioned by the growth habits of the plants. One might anticipate that generally maize and other highly differentiated embryos would show hybrid meristem size advantage more often than plants like tomato in which the embryo is much less extensively developed and in which heterosis has little effect upon organ size. Whaley extended his observations by studying cell and nuclear size changes in the apical meristem during development. Bindloss (8) had presented data on one cross which seemingly sug-

gested that the heterotic hybrids possessed an advantage in nuclear size but no initial advantage in cell size. Kostoff and Arutiunova (81) had previously reported that large hybrids do not as a rule have larger root tip cells than their parents. Whaley found that in all cases development was accompanied by a progressive diminution in size of both cells and their nuclei in the apical meristem. The rate at which this diminution proceeded was, however, markedly less in the hybrids showing heterosis than in the inbred strains. Diminution also proceeded further in the hybrids than in the inbreds. The only other difference noted was in the behavior of the nuclei very early in development. In initial stages of growth the nucleus behaves differently from the cell in that for a short period either during or immediatedly after germination its volume increases. This increase is followed by progressive diminution. In the crosses studied the relative initial increase of nuclear volume was greater in the hybrids than in the inbreds. This behavior suggests differences in the physical biochemistry of the protoplasm, for this initial increase is due probably principally to intake of water. All differences observed indicate that far more than simple size characters are concerned. They rather suggest differences in metabolic level, in the efficiency of the processes of synthesis.

POSSIBLE GROWTH-SUBSTANCE RÔLES

Recent work of Robbins (108, 110) tends to support this view and to give indications as to what some of the factors may be. Robbins worked with fungi lacking the ability to synthesize certain growth substances essential for their own development. *Phycomyces Blakesleeanus* requires an external supply of thiamine or its intermediates for growth. When thiamine and extracts of partially germinated grains of maize were added to cultures of this fungus the cultures receiving extracts from hybrid grains showed greater growth than those to which extracts from the inbred parental grains were added. In the absence of thiamine the hybrid extracts did not have any greater effect than those from the inbreds. *Ashbya Gossypii* requires an external supply of biotin. When extracts from maize grains were added to cultures of *A. Gossypii* in the presence of biotin, the hybrid extracts again produced more growth than those from the inbreds. In the absence of biotin no differences in the amount of fungus growth were observed. Robbins stated that

these facts seem to suggest the presence in the maize extracts of some growth substances other than thiamine or biotin. He called these unidentified substances factor Z, suggesting that Z is a growth-limiting factor which is synthesized in greater amounts by the hybrid than by the inbred lines. Robbins extended this investigation by using seeds of another cross. This time he also used extracts from dry grains. Although the results here differed somewhat, the hybrid extracts were again more effective in promoting growth than those of the inbred parents. To some extent this fact precludes the possibility that factor Z is the result of early growth rather than being active as a limiting factor. The possibility that it is a resultant rather than a causal factor during pre-germination growth still exists. These experiments give some indication that one of the advantages possessed by heterotic hybrids may be the ability to synthesize certain growth substances which the inbreds can synthesize less well if at all.

There is further evidence along another line which lends support to the growth substance idea. Robbins (109) and Whaley and Long (140) have shown that under certain conditions excised roots of hybrid tomatoes and maize grown in cultures show considerably more growth than those of their inbred parents. Robbins, using a cross of inbred lines of *Lycopersicon pimpinellifolium* and *L. esculentum,* found that in solutions supplemented by thiamine, thiamine and pyridoxine, or thiamine, pyridoxine and nicotinamide, the F_1 roots grew more rapidly and produced more dry matter than those of the inbred parents. Robbins was able to show that the roots of P_A showed a greater response to pyridoxine than those of P_B. The roots of P_B showed a greater response to nicotinamide than those of P_A. P_B approached the F_1 in solutions containing the three supplements. Assuming that the growth is limited in all of the lines by the ability to synthesize the three above mentioned substances, and probably a fourth unidentified factor which Robbins refers to White (142) as supposing may be glycine, the data indicate that the hybrid possesses greater relative ability to synthesize pyridoxine, nicotinamide and the unidentified substance. With pyridoxine and nicotinamide the results seem to suggest complementary factors from the parents combined in the hybrid.

Using a solution supplemented by thiamine, pyridoxine and nicotinamide, Whaley and Long (140) obtained essentially the same

results for a heterotic cross between two inbred lines of *L. esculentum.* They were also able to report some significant results with maize, although the excised roots of maize are not particularly satisfactory in culture. In two different crosses early passage cultures (which may show some endosperm influence) showed considerable hybrid advantage. Growth reactions differed somewhat in the different solutions. In the absence of thiamine the hybrid advantage was very small. Growth was best in all strains when thiamine and nicotinamide were added. The addition of these two substances plus pyridoxine limited growth somewhat. Further work with the fungi such as that of Beadle and Tatum (6, 130) promises to yield valuable data concerning the genic control of some growth-promoting substances. It has been found that in *Neurospora* it is possible to induce mutations resulting in deficiencies for specific substances essential to proper growth. Strains with known deficiencies can then be combined.

OTHER PHYSIOLOGICAL FACTORS

Another suggestion of physiological differences appears in the work of Gregory and Crowther (57, 58) who found that some barley crosses inherit greater efficiency in the use of nutrient ions. Whaley's (139) work suggests a more rapid intake of nutrient materials by heterotic tomato hybrids. Others (19) noted that in maize the stomata of certain higher yielding types open earlier in the morning and remain open later in the afternoon than those of lower yielding inbred types. In addition, several investigators have made significant observations on the relation of growth rate to heterosis. Sprague (125) found that in maize the hybrids had a considerably faster growth rate during the early post-germination phases. Kempton and McLane (77) found that heterosis is often, but not necessarily, reflected in embryo size. They also produced evidence similar to Sprague's in finding that the rate of dry matter increase following dormancy in the hybrids was greater than that of the parents but that the advantage was not maintained to maturity.

SEED AND EMBRYO SIZE FACTORS

Any discussion of relation of growth rate to heterosis necessitates an understanding of seed size factors. The two major considerations are the control which the maternal parent effects over seed size

and xenia and metaxenia. Miss Passmore's (101) results indicate the extent of the possible maternal control. This may come about through either nutritional factors or physical limitation by the size of the seed coat. It is important to remember that seed size is not necessarily any measure of embryo size—a point which led to some needless confusion concerning Ashby's data. Xenia and metaxenia have been noted by many investigators. Collins and Kempton (25) found hybrid seeds of maize to be 3% to 21% larger than inbred seeds on the same ear. Wolfe (143) obtained increases up to 16%. Jones (68) reported the same effect in other maize crosses. Paddick and Sprague (99) also reported large hybrid seed size increases. Despite seed size differences the reciprocal hybrid showed uniformity of size at maturity. Ware (134) has reported hybrid vigor in seed weight in cotton. Despite Ashby's contentions, there appears to be little evidence that an initial seed or embryo size advantage gives any lasting superiority. The initial advantage is usually lost before maturity is reached, not uncommonly in the first few days. The same condition seems to exist in certain tetraploids. Fabergé (45) reported a case in tomato where the tetraploid seed weight was 30% greater than that of the diploids. Eleven days after planting the size advantage of the tetraploids had disappeared. Fabergé emphasized that apparently early vigor in F_2 generations may have initial seed weight advantage as its sole immediate cause. Copeland (26) made an attempt to study embryo growth rates and found that some hybrid maize embryos showed added vigor at the 4–10 day stages. This fact suggests that mature embryo advantage is merely an expression of early developed hybrid vigor.

RECIPROCAL HYBRID DIFFERENCES

Whether some of the differences between reciprocal hybrids showing varying degrees of heterosis furnish evidence for cytoplasmic inheritance is still an unsolved problem. In *Lycopersicon* species crosses Schlösser (114) reported reciprocal differences in the osmotic value of the cell sap. His data seem to indicate strict maternal inheritance. Umeya (131) related a peculiar case in silkworms, *Bombyx mori,* showing the influence of nutritional effects. Eggs reared from transplanted ovaries were more vigorous than normal controls. The more inbreeding there had been prior to transplantation the greater was the effect in heightening vigor.

GENETIC BASIS OF HETEROSIS

Returning now to the genetic mechanism which results in bringing about hybrid vigor, it is desirable to consider further the "dominance of linked genes" hypothesis and the other possible explanations of heterosis. The Jones' hypothesis supposes the existence of large numbers of linked "size" genes. The term "size" gene is an unfortunate one. Size is merely the ultimate result of certain developmental processes. What the genes govern is the rate and magnitude of these developmental processes. That there are large numbers of them, linked in various groups, is certainly supported by all the evidence at hand. The hypothesis further supposes at least some degree of dominance of the "large size" genes, the genes producing the faster developmental rates or longer continued processes. On this point there is no experimental evidence. Such a supposition assumes either something in the nature of directional mutation or selection tending to eliminate the unfavorable dominants. The difficulty of detection of favorable mutations tends to obscure the whole matter. Further, the long cultivation, with attendant selection, of maize and other plants showing much hybrid vigor, makes the distinction between "favorable" and "unfavorable" factors a highly artificial one. Collins (24) pointed out this fact in his discussion of Jones' hypothesis. Collins was impressed by the deleterious effects of the many recessive mutations accumulated in maize. It is the action of these deleterious recessives that becomes apparent with inbreeding. Different varieties or strains may be expected to possess different assortments of these deleterious genes. In crossing, all such genes which are not common to both the parents are masked or suppressed by their dominant alleles. The hybrid is then more vigorous by virtue of the suppression of the depressing genes which are operative in the parents. This hypothesis requires fewer assumptions than does that of Jones, although it is not basically any different. It finds support in that the characters uncovered by inbreeding in maize are just such deleterious characters, most of them recessive, as would result in added vigor if suppressed. It does imply though, as does the dominance of linked genes hypothesis, that the majority of surviving recessive mutations must be deleterious, while the advantageous genes are dominant.

Dobzhansky and Rhoades (32) have proposed a scheme for

detecting the presence of favorable and unfavorable genes in maize by the inversion method used for the detection of mutations in *Drosophila*. Their illustration for chromosome I explains the method. By X-ray treatment an inversion is produced in the chromosome which carries the dominant gene P (pericarp color). Homozygous individuals are obtained and crossed with lines or plants recessive for p. The F_1 plants are then either selfed or backcrossed to the homozygous p stock. Any excess of the Pp progeny over the pp ones will be due to favorable growth genes in chromosome I. By crossing the same inversion strain with different inbreds it should be possible to identify those lines having the most desirable complements of chromosome I genes.

Singleton (122) has raised the pertinent question as to whether, after one generation of backcrossing, the differences between the lines compared would be large enough to be revealed, or whether they might not be masked by the heterozygous constitution of the other nine chromosomes. He suggests use of the multiple recessive method for detecting favorable factors.

East (40) proposed a new interpretation of the genetic mechanism, partly in order to overcome the objections to the assumption of dominance of more favorable factors which is essential to the Jones' hypothesis. Citing crosses between "purged" inbreds as showing heterosis, East concluded that although the diminution of vigor accompanying inbreeding is due to the unmasking of deleterious recessives, these genes have little to do with heterosis. It is difficult to see how it would be possible to "purge" inbred stocks. Many of the deleterious genes involved must be ones whose effects are to produce degrees of defectiveness so small as not to be readily detectable. Some degree of hybrid vigor might still result from crossing of the so-called "purged lines" and be due to suppression of recessives. Jones (71) has pointed out that there are many deficient loci in maize after long inbreeding has eliminated all the visible defectives—those in which the deleterious action is readily measured. East then proposed a division of genes into two classes —normal and defective alleles. Since for the most part these combinations of normal and defective genes, as N and n, show complete dominance of the normal and the effect of NN is thus not measurably different from that of Nn, these combinations can not be of importance in producing hybrid vigor. He concluded that

the key to heterosis is to be found in the behavior of normal genes which control the inheritance of quantitative characters. East repeatedly drew division lines between classes of genes, setting off the qualitative genes from the quantitative ones and those affecting morphological pattern from physiological pattern. This arbitrary distinction is useful in some respects but it creates considerable confusion and obscures his explanation of the heterosis mechanism. He assumed that there are great numbers of different alleles among the normal or nondefective genes, the group which primarily controls the physiological efficiency of the organism, the set of characters related to heterosis. All evidence to date indicates an absence of any considerable degree of dominance in so-called quantitative character genes and suggests rather that each member of an allelic pair exerts an effect upon the ultimate character expression. East suggested, therefore, an hypothesis which combines part of Jones' linked gene theory, part of Rasmusson's (104) quantitative factor hypothesis, and the old idea that heterozygosity is in some way concerned in heterosis. His idea may be stated briefly as follows: size traits are controlled by a large number of genes in various linkage groups; among these genes dominance is virtually nonexistent but there are numerous multiple allelic series; if in a given series each member affects a different physiological condition, then the heterozygous condition may be expected to produce cumulative results; that is, if a_1 affects a somewhat different process than its allele a_2, a_1a_2 may have a greater effect than a_1a_1 or a_2a_2. This hypothesis implies some sort of complementary action between alleles—a_1 supplies what is lacking in a_2, or *vice versa*. East did get away from the dominance issue, an advance, since, as pointed out above, there is little good evidence for even partial dominance of larger size genes or more favorable growth genes. However, the essential assumption that the mutated alleles of a series affect different processes which in combination produce some kind of complementary or cumulative effect, is without much evidence to support it.

Jones (69) did find possibly advantageous allelic differences from parental types in heterozygotes of *Nicotiana*. Dunn (36) has reported a case of lethal alleles in mice acting in such a way as to permit viability in the heterozygotes. Alleles, with such an additive effect as East supposed, have been found in *Godetia* (62).

However, there is not much ground for suggesting that these interactions play any considerable rôle in heterosis.

Significant contributions to the theory of the genetic mechanism responsible for heterosis appear in recent work by Dobzhansky and Sturtevant and their co-workers (30, 31, 33, 128, 129), which is not primarily concerned with the heterosis problem. Working principally with *Drosophila pseudoöbscura,* these investigators have shown that in natural populations there are large numbers of more or less unfavorable recessive mutations. Cross fertilization permits accumulation of these up to an equilibrium point which is determined by the mutation rate and the population structure. Where the population size is of sufficient magnitude there exists a great variety of chromosomes with a diversity of gene content. In such populations cross-bred individuals greatly exceed the homozygotes in vigor by virtue of suppression of unfavorable recessives. Jones (71) has clearly recognized the importance of this type of gene action in producing vigorous hybrids. An explanation of some hybrid vigor on the basis of such gene action rests upon the same fundamental principle as that of the original hypothesis of G. H. Shull and Jones, but it does permit a somewhat different interpretation of the phenomenon.

If heterosis is due to the suppression of unfavorable genes and not to some special superiority on the part of the dominants, a change in the conception of what heterosis constitutes is necessary. The literature is pervaded by the idea of hybrid vigor as something better than the "normal", if the term is permissible. The hybrid is thought to have an absolute advantage. This view has always seemed curious when one considers what happens during continued inbreeding in a plant like maize. If the suppression hypothesis is accepted it is to say, in effect, that the hybrid represents the "normal" or biological optimum under a given set of conditions and that the parents, whether purposely inbred or not, are inferior types—they contain an accumulation of deleterious factors. Such an idea certainly fits the facts of maize breeding.

An examination of this hypothesis in the light of what is known about heterosis adds weight to its validity. Arbitrarily the deleterious genes may be arranged in a descending scale of effectiveness as: lethals → semi-lethals → powerful developmental modifiers → weak developmental modifiers. Dobzhansky has emphasized the

existence of all these classes of factors in his material, and the general body of evidence points to their presence in all mutating organisms. Since their effect is one of complete elimination, lethals are of no interest in relation to heterosis. The other factors, and particularly their degree of effectiveness, are, however, important.

Plants may be divided into three groups—those which are normally completely cross-pollinated, those completely self-pollinated, and those in which both cross- and self-pollination take place. In the cross-pollinated types, except under certain conditions, an accumulation of semi-lethals and both the powerful and weak developmental modifiers may be expected. This accumulation is conditioned by the mutation rate and by the size of the effective breeding population. A low mutation rate would limit the number of deleterious factors accumulated, but time must also be taken into consideration. Effective population size is an equally limiting factor. If the breeding population is so small as to permit frequent union of gametes carrying the same deleterious factors, then the rate of elimination will be high. Any considerable accumulation of these factors can take place only in populations large enough to contain many different chromosomes and not have them eliminated by frequent *like* gametic combinations.

In self-pollinated plants the effective population size has been reduced to the minimum. Immediate elimination of any mutation with a considerable detrimental effect is a certainty. Only those mutations whose detrimental effect is weak enough to allow them to persist without being suppressed by normal alleles can accumulate. These plants can accumulate, then, only those mutations which fall in the class of weak developmental modifiers. And these can accumulate only to the point where their combined effects begin to produce elimination. In plants where self-pollination is not obligate, the population size is somewhat larger. Its exact size is determined in part by the relative proportion of self- and cross-pollination. Here if the population size is large enough, the more powerful of the developmental modifiers may also accumulate. In very large effective populations even a few semi-lethals might be found.

Any actual measure of comparative amounts of heterosis is, of course, not possible. However, it is of considerable interest to obtain a general idea not only of the degree of added vigor and the consistency with which the phenomenon occurs in any given organ-

ism, but also in what type of organisms it is most frequently encountered. Engledow and Pal (44) made a survey of what they considered well authenticated instances of hybrid vigor and came to the conclusion that it occurs most often in crosses between plants which are diploid, normally cross-pollinated and endospermic. Reliable numerical data are few, and no standards of comparison exist, but in the general body of literature the following facts stand out. Hybrid vigor is exhibited by organisms of all classes. It is found in *Lycopersicon, Phaseolus* and *Soja* where obligate self-pollination is approached; the cereal grains, which range from highly self-fertile, completely self-pollinating forms, as in certain ryes, to normally more or less completely out-crossed forms like maize. Completely self-sterile plants as certain nicotianas show it when crossed. It has been pointed out several times by various investigators, notably East (40), that within certain limits the amount of heterosis varies directly with the genetic disparity of the stocks. Unrelated lines show more heterosis than related ones. Karper and Quinby (75) have even attempted to use the amount of hybrid vigor as an index of the degree of relationship between parents. Inbreeding prior to crossing tends to greatly increase the amount of heterosis in heterogamous plants. The limit to the increase is the point where, as East (40) has expressed it, "the genic differences fail to nick". To a certain extent it follows that the amount of heterosis will increase as obligate cross-pollination is approached, since the basis of greater genetic disparity is laid more rapidly in an out-pollinating population if it is not too small. Controlled inbreeding heightens this disparity.

For example, in maize, particularly in purposely inbred lines, the yield of the hybrids is often many times greater than that of the inbred parents and may exceed that of the original heterozygous individuals by more than 50%. Further, the relative number of crosses showing hybrid vigor is great. In tomato, on the other hand, the hybrid advantage in yield rarely reaches 50% (Alabouvette and Titard (1) give a range of 25% to 40%), and heterosis occurs only once in a number of crosses. It would appear, then, that heterosis is more prevalent and hybrid vigor greater in those forms whose method of reproduction permits accumulation of the more deleterious mutations and, it might be added, where selection has heightened this accumulation. Heterosis is less outstanding

and a much more elusive phenomenon in those forms whose method of reproduction eliminates all except the less deleterious genes.

Heterosis in maize is worth further consideration both as maize is the type of organism in which hybrid vigor is most manifest and because it is the best known and so far most economically valuable case. Fisher's (46) genetical theory of natural selection has a direct bearing upon the possible explanation of heterosis in organisms like maize. Fisher maintained that in a population where heterozygous individuals predominate, the heterozygous condition will be optimum for many pairs of genes. Concerning population size in maize we have no experimental evidence. However, maize is wind-pollinated and grown in cultivation over large areas. These two facts indicate an effective population size much greater than that of self-pollinated or specific insect-pollinated plants. The very nature of progressively inbred lines of maize is certainly evidence enough that inbreeding uncovers large numbers of deleterious recessive genes. There is proof, too, that these genes range from slight developmental modifiers to lethals in the fact that generations of inbreeding usually produce strains weakened to various degrees.

Jones (70) has recorded the results of a long continued inbreeding program in maize. Of four original lines, one line and one second generation separated sub-line failed to survive. The surviving lines were selfed for 30 generations. Reductions in height were encountered in each of the first five generations, yield reductions in the first 20 generations. Jones' data also give evidence of continuing mutation, since sub-lines separated at different times showed transmissible variations in some instances and not in others, Jones has noted that none of the variations which appeared during the whole period of inbreeding was such as could be interpreted as favorable to survival. It must be borne in mind, however, that, where hybrid vigor is concerned, it is the expression of the character in the hybrid rather than in the inbred which is important. Singleton (122) and others have stressed that the only way to test the merit of an inbred is by crossing.

Supposing it were possible to start with two maize plants which were at least to a certain degree homozygous, crossing these plants would produce a heterozygous F_1:

$$\begin{array}{ccc} \text{AAbbCCdd} & \times & \text{aaBBccDD} \\ & \text{AaBbCcDd} & \end{array}$$

If the recessives were deleterious even to a limited degree, then the hybrid would be superior to either of the parental types. Any heterozygous individual would be superior to a homozygous individual, since if there were any considerable number of factors involved, an individual approaching homozygosity would possess certain of the less favorable recessives. Superiority in any number of traits or characters would most likely include some which give a selective advantage.

Fisher (47) has made the suggestion that in certain cases there is an avoidance of homozygous dominants. He calculated that in *Paratettix texanus* some 40% of them were eliminated in each generation, supposedly because of an accumulation of unfavorable factors made possible by some selective advantage favoring the heterozygotes. In maize certain dominant mutations, such as Rg, ragged leaf (12), and Tp, teopod (83), are known to have much more deleterious effects in the homozygous than in the heterozygous condition. It may be supposed that other genes with less obvious effects might operate in the same manner.

The way in which maize culture has been handled, even by the Indians, would seem to indicate that, consciously or not, there has long been a process of selection favoring the heterozygote. Thus it is perhaps possible that some of the heterosis in maize may result directly from heterozygosity rather than from the action of dominant genes either in the sense of "more-favorable" factors or as recessive suppressors. With these genes, then, the distinction between recessives and dominants is non-existent or unimportant. It ought to be pointed out that this possible importance of heterozygosity is not based upon the supposition of an initial germinal stimulation but upon a specific type of allelic interaction. The mechanism is similar to that proposed by East (40). It is not, however, based on complementary action of alleles affecting different processes, but upon the assumption of greater advantage, perhaps physiological efficiency, for the heterozygote, arising from the fact that certain alleles may have less of a weakening or other unfavorable effect when heterozygous than when homozygous.

Heterozygosity rather than dominance has always been a rather appealing possibility for explaining maize heterosis. Stubbe and Pirschle (127) cited the behavior of a certain mutant race of *Antirrhinum majus* as evidence in the support of the heterozygosity

theory. They obtained a race with a chlorophyll deficiency and a tendency to mutate back to the normal in both the somatic and generative tissues. Upon selfing, variable numbers of phenotypically normal individuals were obtained. These individuals were heterozygous with respect to the mutant gene. They exhibited a distance degree of hybrid vigor. The authors maintain that this case constitutes proof that heterosis is due to heterozygosity rather than the additive effects of dominant factors.

On the other hand, other suggestions presented lead away from the heterozygosity idea (105, 107). These investigators have developed the so-called "convergent improvement" system in maize. In this work the F_1 hybrid is backcrossed to each parent through several generations, while selecting in each generation for the favorable characters of the non-recurrent parent. The inconclusive data suggest that hybrids between partly recovered lines may be more vigorous than those between the original parents, even though they are less heterozygous. Murphy (98) has more recently secured hybrids from converged lines which yielded more than the original F_1 cross. This suggests incomplete dominance of the greater yield factors in the F_1.

Singleton (123) has reported what he interprets as a single factor for heterosis. Purdue 39, a widely used sweet corn, when inbred mutated to a dwarf form, C_{30}. The progeny from crosses between P_{39} and C_{30} indicated that a single recessive mutation was involved. Crosses of the mutated form with other inbreds, C_{13} and C_{15}, sometimes produced hybrids giving greater yields than crosses between P_{39}, the normal form, and these same inbreds.

Powers (103B) has recently proposed what he terms an expansion of Jones' theory for the explanation of heterosis. From earlier investigations (103A) Powers concluded that actually heterosis and dominance are expressions of the same genetic phenomenon. Applying the term "heterosis" to a favorable end result such as yield under a given set of conditions he was able to show that although the multiplicative effect of a given pair or set of genes results in heterosis, some of the individual genes may show dominance for an unfavorable character, such as smaller fruit size. He points out that there thus may be no dominance, or partial dominance of "unfavorable" genes and still be heterosis for a given character if the gene action is multiplicative. By his association of heterosis

and dominance Powers emphasizes a point of importance, for if the two are degrees of expression of the same physiological mechanism, then, as there is dominance of both favorable and unfavorable alleles there must be a condition of "unfavorableness" balancing what Powers has termed "heterosis" and for which this paper has used the less scientific term "hybrid vigor." Although little studied this condition has been recognized by several investigators and is generally referred to as "negative heterosis." Powers proposed "non-beneficial heterosis," a much better term, since in discussing the phenomenon we are limited, so far at least, to a consideration of end results beneficial in an agricultural or horticultural sense rather than in relation to natural conditions.

Mather (94) has made some interesting suggestions concerning heterosis in a recent review paper. He points out that survival under natural selection hinges upon proper genetic balance within the organism. In homozygous inbreeding organisms this balance is mainly an internal one—among individual gene combinations. In outbreeding organisms, such as maize, this internal balance is over-shadowed in importance by relational balance among pairs of chromosomes working together. Intercrossing brings together different polygenic combinations. If these combinations have not been selected for good relational balance, the hybrid will show a greater or lesser departure from the optimum for the conditions under which the organism exists. Hybrid vigor is the designation given to such a departure when it is in the direction of increased size. It follows that since hybrid vigor is a sign of poor adaptation it will be selectively disadvantageous under natural conditions.

Mather highlights the already apparent relation between artificially imposed conditions and the occurrence of hybrid vigor. He points out that heterosis is the automatic consequence of diminution in the freedom of interbreeding among strains, and an effective agent in the development of isolating mechanisms and hybrid sterility.

A few cases which may actually be produced by factors other than heterosis deserve mention. In beans (91), soybeans (132) and rye (11) there have been instances in which hybrid vigor appeared to be carried beyond the F_1 generation, even into the F_3 and F_4 in the beans. It remains for such cases to arise under very

carefully controlled conditions before any detailed consideration is necessary. Some minor cultural variation from one year to another might easily give rise to the condition. It is probable that cases appear in which the homozygous condition for some factors is superior to the heterozygous or in which large numbers of the advantageous genes are grouped in individual F_2 and F_3 segregates.

POLYPLOIDY AND HETEROSIS

The significance of polploidy in relation to heterosis has been studied but little. Huskins and Smith (65), who recognized the implications of Fisher's suggestion (46) that under certain conditions the heterozygous state may be superior to the homozygous one, have discussed the matter briefly. They supposed a specific type of hybrid vigor to result from certain combinations of alleles in all cases. They assumed that in an obligate cross-fertilizing organism there is added to this specific action a more generalized one dependent upon small differences between many alleles. An autogamous allopolyploid should exhibit the specific type of hybrid vigor of its diploid ancestor. An allogamous polyploid should have this specific type of hybrid vigor plus the generalized type. It would seem difficult to separate the two types of hybrid vigor. Degree of the differences will be dependent upon the number and nature of the accumulated mutations. The apparent specificity of the phenomenon in autogamous organisms is conditioned by the fact that a certain degree of dominance is operative. In the allogamous organism, if, as has been supposed for at least some cases, the heterozygous state is superior, dominance and recessiveness have disappeared, their absence makes the detection of specific differences difficult—hence perhaps the appearance of a generalized effect.

East (40) came to the conclusion that natural polyploids do not show hybrid vigor. This would mean either that amphidiploids do not possess heterosis or that natural polyploids do not originate from hybrids. Artificial polyploids, according to East, often do show hybrid vigor. He cited the case of *Spartina Townsendii* which is an amphidiploid from a cross between *S. alternifolia* and *S. stricta.* So vigorous is the polyploid that it has replaced the parental forms (64). Other such forms are *Primula kewensis,* a turnip × swede cross (51) and *Nicotiana glutinosa* × *N. tomentosa* (54, 42).

A word should be said about hybrid vigor in organisms other than

the green plants. Dickson (29) reported what he termed "hybrid vigor" in one cross between strains of *Neurospora crassa.* Since the mycelial growth is haploid the term is hardly applicable. However, Dodge (34) has reported in *Neurospora* a very interesting phenomenon which is perhaps related to heterosis. He combined strains of *N. tetrasperma* in which nuclear migrations occurred so that nuclei of both strains came together in a common heterocaryotic mycelium. In certain such "crosses" the heterocaryotic mycelium grows much more rapidly and abundantly than that of either component. Dodge termed this phenomenon "heterocaryotic vigor" as distinguished from "segregant haploid vigor" (as reported by Dickson) and from the true "hybrid vigor" of diploids.

HYBRID VIGOR IN ANIMALS

Earlier data on hybrid vigor in animals have already been mentioned. Some consideration of later recorded cases is warranted to show the universality of the phenomenon. Generally speaking, however, hybrid vigor has been less studied in animals than in plants. It has been reported in most of the laboratory animals which have been studied genetically. Marshak (93) found it in mice—with considerable difference in degree in reciprocals. He attributes the reciprocal differences to genome-cytoplasm reactions. Little and Tyzzer (86) had earlier reported heterotic F_1 mice in which tumors grew at a more rapid rate than in sib-crossed lines. Castle (14) interpreted this as being due to the heterozygosity of the F_1 mice. Grüneberg (59) recorded what he believed to be the largest recorded litter of mice—19, with an average birth weight of 1.54 gms.—in hybrid mice. Green (55, 56) had reported several cases where hybridization increased litter size. Fortuyn (49, 50) obtained like results. Strong (126) made an observation similar to that of Little and Tyzzer by noting that transplanted tumor tissue grew about 25% faster in heterozygous F_1 mice than in the parental homozygous inbreds or the F_2 or F_3 individuals. Vicari (133) suggested that the faster maze learning exhibited by F_1 hybrids between *Mus musculus* and *M. Wagneri* might be due to heterosis. Whether heterosis is actually active in instances such as these can not really be determined until we know more about specific genic effects. Livesay (87) found added vigor of varying amounts in different rat crosses. In hybrid vigor occurring in crosses between

guinea pigs and *Cavia Cutleri,* Castle (16) drew an important distinction between operation of specific size factors, which in this case show incomplete dominance, and heterosis. Wright (144, 145) also observed heterosis in guinea pig crosses. Numerous observations are recorded relating to the presence or absence of hybrid vigor in rabbits (15, 17, 100, 103). There are several cases among animals of economic importance. Roberts and Laible (111) reported a case in swine which has been interpreted many times as showing the establishment of early size differences resulting from heterosis. They double mated a Duroc Jersey sow first to a Poland China, and then to a Duroc Jersey boar. At birth the cross bred pigs were significantly larger than the pure breds. At six months the crossbreds were much heavier. Whether this is really a case of hybrid vigor is not clear. Others (115) made the reciprocal of this cross, mating a Poland China sow first with a Poland China and then a Duroc Jersey boar. They found the inbreds to be heavier at birth, but the hybrids gained weight more rapidly after birth. Still others (147) have noted sheep crosses of Merinos × Hampshiredown which gave progeny of higher than the purebred birth weight, and faster post-natal growth. Cattle-yak hybrids have been subject to several investigations. Lus (90) reported F_1 hybrids heavier and generally larger than the parents. Kushner (82) found that such hybrids greatly exceeded their parents in the blood characters of Hb content, number of erythrocytes, and alkalinity. This condition makes for greater oxidizing power which in turn produces more intense metabolism and more rapid growth. Kushner reports similar findings in hybrids between dromedaries and bactrians, and in the mule, which was accepted by all the early writers as the classic animal example of hybrid vigor. Heterosis has been noted also in Zebu × Yak crosses (146).

In fowl hybrid vigor has been reported in many cases. Dunn (35) noted the greatest manifestations as being the hastening of sexual maturity. Jull (73, 74) found hatchability and vigor to be increased by crossing. Others (137, 138, 148) have pointed out that hybrid vigor occurs in chicken crosses only in cases where the parent stocks are very homozygous. Warren (135, 136) crossed Jersey Black Giants and White Leghorns and found that the F_1 hybrids were better egg producers, had lower embryonic and chick mortality, and grew faster. It is of interest that none of the reports

of heterotic chicken crosses indicates that the hybrids are more than intermediate to their parents as to mature size.

Among insects hybrid vigor has been reported in the silk worm, *Sericaria mori* L. (9). Pearl, Parker and Gonzalez (102) proposed that increased life span in certain *Drosophila* crosses may be a manifestation of hybrid vigor. Robertson (112) suggested the possibility that hybrid vigor is concerned in parthenogenesis in the Tettigidae. He supposed that heterosis might possibly stimulate cleavage in the egg early, thus beginning the development of an embryo.

To the case in fishes mentioned above can be added certain intergeneric sunfish crosses (63). Hybrids of *Apomatis cyanellus* × *Eupomatis gibbosus* greatly exceeded either parent in size and weight.

CONCLUSION

Any attempt to arrive at a single definitive explanation of the genetic basis for the phenomenon of hybrid vigor seems unwarranted with the evidence at hand. As Lindstrom (85) has pointed out, there are no critical proofs for any of the so-called "heterosis theories". Some hybrid advantage is undoubtedly due to simple factor combinations—complementary gene action as usually understood. Much of it is due to the suppression of deleterious recessives in the hybrid, or the favorable action of dominants. The interpretation of the concept in this case turns on the degree of deleteriousness assigned to the recessives. Stated either way it means that the dominant genes concerned are relatively more advantageous to the organism than their recessive alleles. Heterozygosity of certain alleles may account for some of the hybrid superiority where mutation and selection have brought about a degree of advantage associated with the heterozygous condition. Any or all of these operating mechanisms can bring about hybrid vigor in the progeny of a cross.

The manifestations of heterosis depend upon the factors involved, and if elevation of the general metabolic rate is concerned, on the growth form and characteristics of the organism. The fundamental processes responsible for the production of hybrid vigor may differ somewhat in various organisms, but all of them are factors which increase the physiological efficiency of the hybrid organism. The end effect is a general one, although the immediate effect may be

as specific as the slight increase of a given substance at one stage in development.

Further, it should be kept in mind that hybrid vigor is a phenomenon observed and studied, for the most part, in organisms under domestication. Like other effects its level may be supposed to differ in response to changes in environment.

As Smith (124) has stated in an excellent recent review of quantitative inheritance, solution of the heterosis problem awaits the presentation of much more evidence concerning the general action of genes governing size.

Much knowledge regarding the phenomenon of heterosis should be derived from the present day genetic studies aimed at investigating gene action in controlling physiological reactions and their interrelations in various organisms under carefully controlled conditions. In problems of developmental genetics the background of hybrid vigor offers a more fertile field for study than ever before.

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SPORES AND POLLEN AS MICROFOSSILS

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INTRODUCTION

Spores and pollen have long been known as fossils, but it was not until approximately thirty years ago, when statistical methods were applied to the fossil pollen of European peats, that a wide interest became apparent. By statistical methods it is possible to determine much concerning the development of earlier vegetation, and from that to derive other information of scientific and cultural value. Interest in the older fossils has been rekindled, but as yet these have not received the attention given the pollen fossils of Quaternary age.

The term "plant microfossil" is used here for spores and pollen grains of plants irrespective of their affinity. Algae, fungal hyphae, trichomes, certain leaves and other plant parts that might come under the heading of microfossils are not included in the present discussion.

The literature pertinent to plant micropaleontology is widely scattered through periodicals of many sciences. It is to be found not only in publications of botany and geology, but also in those of zoology, chemistry, physics, and those of economic interest.

OBJECTIVES OF THE STUDY OF PLANT MICROFOSSILS

There are three major objectives in the study of plant microfossils: (*a*) to establish the biological affinities of isolated fossil spores and pollen, and to determine their phylogenetic relationships; (*b*) to use spores and pollen as indices in the stratigraphic study of rocks; and (*c*) to aid in the understanding of paleogeographic and paleoecologic problems.

(*a*) There are thousands of unassigned species of spores and pollen existing as isolated bodies in shales, coals, clays and peats. It is not conceivable that all spores and pollen ever will be assigned to their natural species, since there are similar forms in widely divergent groups, and also, as in other biological phenomena, similarity of form increases with genetic proximity. Very frequently in modern plants, species within a genus are not distinguishable from each other on spore or pollen characters, and it can be safely assumed that

this is also true for ancient plants. Certain extinct species of Tertiary plants have spores and pollen which closely resemble existing species. Such a condition complicates micropaleontological problems, but some of these may be solved with the completion of extensive morphological studies. When these investigations are well under way, it may be that a combination of micro- and macrofossils will be used in the delimitation of various genera or species. In the modern heaths, for instance, it is difficult to separate certain genera on the basis of pollen, but because the leaf trichomes are quite diagnostic, it is frequently a simple matter to determine the identity of some of the fossil heath pollen that are encountered in postglacial sediments. In the older rocks it may be possible in the future to distinguish certain species of *Lepidodendron* by a combination of leaf and spore fossils.

In the pursuance of fossil morphological and evolutionary studies, it will be necessary to learn a great deal more about modern spore and pollen morphology. Though numerous studies of fossil fructifications have been made, they represent only a beginning of a very necessary part of the chain of knowledge leading to the maximum usefulness of microfossils. To illustrate the type of studies needed, papers on the structure of *Rhynia* (35), on the morphology of the pollen in *Cordaites* (23) and on *Senftenbergia* (62) may be mentioned.

(*b*) The present use of plant microfossils in stratigraphy lags far behind the use of animal microfossils, though both were discovered at approximately the same time. Several reasons account for the difference, but the most important appears to be the lack of knowledge concerning the identity of the plant fossils. Such knowledge is now being pursued, and the results already attained from the late Paleozoic, Mesozoic and Cenozoic rocks testify to the stratigraphic value of plant microfossils, and indicate the development of a useful new adjunct to this field of geology.

Animal microfossils, such as the Foraminifera, sponge spicules and ostracods, occur widely through the limestones, shales, fine sandstones and their clastic equivalents. They do not occur very frequently in coals or peats. On the other hand, plant microfossils occur most abundantly in coals and peats, less frequently in limestones, shales and fine sandstones. Therefore, it would seem that together the two would complement each other in a thorough study of sedimentary rocks.

In pre-Quaternary rocks the emphasis of plant micropaleontology has been placed upon stratigraphic value, an interest that has not been fully explored by workers in the clastic sediments of the Quaternary period. In the latter, the time intervals have been relatively short, and we still lack a complete understanding of the glacial drift pattern. These factors, coupled with the problems of forest succession and probable climatic shifts, presents variables that will require years of intense research to comprehend.

(*c*) The extent to which plant microfossils have been used in determining the paleogeography and paleoecology of plants diminishes rapidly with the increased age of the rocks and decreased knowledge of plants. The science of pollen-statistics, as known to most people, has dealt mainly with glacial and postglacial sediments. Great emphasis has been placed upon the determining of forest succession, climatic shifts and plant geography. Results from these studies have reached rather more definite conclusions in Europe than elsewhere.

Attempts to discuss geographic and ecological problems in pre-Quaternary microfossil deposits are exceedingly few. Wodehouse (95) and Simpson (75) have described in some detail the plant ecology of the Eocene from deposits of oil shale and coal. Back beyond the Tertiary the problems of paleobotany multiply rapidly, and the lack of specific knowledge of spores and pollen greatly handicaps the development of these phases of the science. The diagrams of Raistrick (64) and others definitely show that plant succession occurred in the ancient Carboniferous forests, and the prospects of exploring this field are by no means hopeless. The marked similarity of fossil spores and pollen between Europe and North America is an indication of widely spread similar or identical species. The use of microfossils to trace ancient forest migrations seems possible, and this information will be of real value to botanists and geologists alike. If the paleogeographic and paleoecologic phases become sufficiently clear there is reason to believe that they can be used in solving such problems as the Pennsylvanian cyclothems, the separation of late Mesozoic and early Tertiary formations, and the many problems of climatic sequence.

METHODS OF STUDY

The numerous laboratory techniques employed by investigators of peat fossils have been brought together and described in detail by

Erdtman (21). The methods of other paleobotanists have not been collected in this manner, and consequently must be sought throughout the literature. Field practices are generally not very well described in botanical literature, but those of the animal paleontologist are in a large part applicable to paleobotanical problems, and the book by Camp and Hanna (11) on "Methods in Paleontology" will be found useful. General procedure and methods are given below in brief form.

Collecting. In this phase of the work the type of sedimentary rock must be considered, for each requires different collecting methods. From consolidated rocks such as shale and coal the samples are taken at outcrops or from borings. The clastic sediments may be collected by direct digging or by boring with a soil auger or other type of sampler. Sand and clay samples may be recovered with well-drilling equipment, or if from lake sediments, by equipment designed by Ira T. Wilson (59). Peat and marls are collected by the use of the Davis (7) or Hiller (21) sampler.

The methods of sampling may be put into two general classes, channel and interval sampling. The former consists of cutting a block or column vertically through the deposit. The column then may be treated as a unit, divided into intervals of regular lengths, or divided into intervals determined by lithological benches. The use of lithological benches is frequently employed when coals contain pyrite or shale partings. Peat and silt can be collected easily at definite vertical intervals and are usually studied in that manner.

Preparation. The common methods of preparing plant microfossils are: (*a*) isolation, (*b*) thin sectioning, (*c*) nitrocellulose peels, (*d*) maceration, and (*e*) dispersal.

(*a*) Isolation. Frequently, large spores may be taken directly from certain coals, soft shales and glacial drifts. This is especially true of the large Devonian spores and the megaspores of the Pennsylvanian coals. Where considerable detrital material exists, separation may be accomplished by screening or by the use of a separatory funnel and bromoform (11). In the latter method the heavier materials sink to the bottom of the funnel and the spores float. Sometimes it is necessary to repeat the separation several times in order to isolate the fossils. Where large spores are being isolated from unweathered bituminous coal it is necessary to either pulverize or macerate the coal first and then proceed with screening operations.

(*b*) Thin sectioning. This method is well known. Briefly, the procedure consists of cutting a small block of coal or other rock, polishing one surface very smooth, cementing this face to a glass slide, and finally grinding the specimen to a desired thickness on a glass plate with carborundum grit. The method has been fully described by Thiessen (*79*). This manner of studying microfossils is frequently desirable when a comparison is being made of the various rock components and when morphological problems are being investigated.

(*c*) Nitrocellulose peels. This method has been applied largely to coal-ball studies, but has also been used on coals by Darrah (15), and by the writer on oil shales. The method consists of cutting the desired section of rock, polishing it smooth with carborundum powder, etching the surface with dilute acid, drying it carefully in warm air, applying one or more coats of nitrocellulose preparation to the surface, and when thoroughly dry, stripping off the peel and mounting it in balsam or other medium for microscope study. The greatest value of the peel method to microfossil studies is undoubtedly in coal-ball investigations. In these concretions there is a wealth of spore and pollen material that can be readily studied with peels. The method is especially suited to morphological studies, since one can frequently secure sections of spores or pollen by carefully grinding and etching the rock surface. Spore structures such as elaters are lost in maceration procedures but are retained by the peel method.

(*d*) Maceration. This is now the most common method of preparing consolidated sedimentary rocks for plant microfossil study. Various chemicals have been employed, depending upon the type of rocks studied. Sedimentary rocks containing calcium carbonate can be disintegrated by various strengths of hydrochloric acid, and those of siliceous composition are treated with hydrofluoric acid. Coals must receive special treatment depending upon their rank and state of weathering. Bituminous coals that are cut from a recently opened seam usually need treatment in Schultze's or other such macerating solution for a week or longer, but weathered coal macerates more easily. When the preliminary softening has taken place the coal is washed free of the solution, covered with a dilute solution of ammonium hydroxide and allowed to stand for several hours. Again the material is washed with water, and is then ready for study.

Coals react diversely in macerating solutions, and care must be taken to prevent being burned if the reaction is violent. The material should be frequently stirred and occasionally the progress of the maceration should be inspected by examining a sample of the coal under the microscope. Over-maceration of the spores and pollen readily occurs and will frequently destroy the diagnostic characters of spore or pollen walls. Maceration methods have been described (43, 85).

Some of the weathered coals and brown coals can be easily macerated with weak ammonium hydroxide, after which they must be washed with water until free of the odor of ammonium. Centrifuging the material greatly reduces the time of preparation, but care must be taken, for the fossils are easily broken.

(*e*) Dispersal. Unconsolidated sediments yield their spores and pollen quickly when dispersed by chemical means. Many methods have been published, but the most common now in use seems to be the KOH method described by Erdtman (20). It consists of boiling the peat sample in a 10% solution of potassium hydroxide, screening the humic mass, and concentrating the finer material by decantation or centrifuging. A second method has since been developed by Erdtman (21) which is much more drastic, but a greater concentration of microfossils is attained.

Staining. Most of the older microfossils do not require staining in order to be studied or photographed, for they are usually of various shades of brown or yellow. Fossils younger than the Paleozoic usually need to be stained in order to get the best photographic results. Staining of fossils in silt sediments also aids in readily distinguishing them from the inorganic particles. The stains most often used are safranine and gentian violet.

Mounting. There are several methods of mounting plant microfossils, but the two most frequently in use are the glycerin jelly and diaphane methods. The former is in some cases not as satisfactory as the latter, but its simplicity makes it possible to prepare a large number of samples in a very short time. Diaphane has been used with considerable success for permanent mounts of microfossils. It has the advantage of being somewhat more transparent than glycerin jelly. Other media that have proved useful are balsam, water-glass and Karo syrup. The last two should not be used if the material is to be kept over a period of years, for the media tend to become crys-

tallized. Very large spores may be mounted dry on slides that are especially constructed for Foraminifera (11).

Photographs. The variety of plant microfossils is so numerous that if only line drawing illustrations are made, the task becomes very laborious. It is therefore desirable that some quick and inexpensive method of photographing be developed. Several types of cameras are available for this work, but if one must use other than 35-mm. film, this part of the work is very expensive. The film known as Panatomic X gives very satisfactory results, and when developed with a contrast developer such as D–11 or D–8, it is possible to enlarge the photographs to almost any size. Photographs of plant microfossils mounted on standard filing cards with information concerning the source of the material, the geological age, and a morphological description of the fossils, *etc.*, become of increasing value as the collection of microfossils grows. These photomicrographs become an absolute necessity when any extensive work is undertaken.

Classification. As in other branches of natural science there is a need for a clear understanding of taxonomic practice. Various systems of classification are now in use among plant micropaleontologists, but it is natural to expect that the system that attains the greatest proximity with phylogeny will be accepted. Some of the problems of classification will be discussed under the headings of various geological ages. However, it is necessary to state here that if order is to come out of the maze of taxonomic difficulties now existing in microfossil literature, there must be adherence to an international system of nomenclature.

MICROFOSSILS OF PALEOZOIC AGE

The rocks of the Lower Paleozoic era do not contain an abundance of plant spores, and those that are known must be considered with some degree of uncertainty. The earliest plants to produce spores were aquatic, and it is probable that the fossil spores reported from the Cambrian and Ordovician periods are all of that type.

Upper Cambrian spores with tetrad triradiate scars characteristic of the pteridophytes have been reported by Darrah (14) for the Ost-Gotland Swedish oil shale. If the spores prove to be Cambrian in age, they are the oldest showing that type of scar.

In the Ordovician shales of Illinois and Iowa, dark spore-like

bodies are frequently observed. These have, to date, received little attention and their status is still questionable.

At present our understanding of Silurian spores is, like those of Ordovician age, very incomplete. However, since the discovery of Silurian fossil terrestrial plants in Australia, we can expect eventually to find a richer assortment of plant microfossils.

Devonian rocks contain numerous plant microfossils of which the best known type is a large dark-colored spore called *Sporangites.* It is a flattened disk-like body of about one millimeter in diameter. Frequently this fossil occurs in great quantities in the black Devonian shales of the Middle West and of Ontario, Canada. It is also known from Europe, South America and Australasia. The name *Sporangites,* as set up by its author, is difficult to retain, except as a term of rough description for dark, rounded, spore-like bodies. The name was first used by Sir Wm. Dawson (17) when he described some Devonian fossils as *Sporangites Huronensis.* At that time he considered them as the spores of *Lepidodendron,* but several years later, while describing two other species (18), he established the genus *Protosalvinia* and transferred *Sporangites Huronensis* to it. Dawson then supposed the spores to be related to the water fern, *Salvinia.* Today it is known that such a relationship is not the least probable. In 1875 Newton (50) described the Tasmanite and Australian white coal. Here he found spores similar to those of the American Devonian shales. The fossils were named *Tasmanites punctatus.* In the course of the descriptions of *Sporangites* and later of *Protosalvinia,* ambigous definitions were given. In an attempt to straighten out the situation, Schopf, Wilson and Bentall (71) have found it desirable to discard the name *Sporangites* as a *nomena ambigua,* and use Newton's *Tasmanites* for the large, thick-walled punctate spores of the Paleozoic rocks. The name *Sporangites* is retained as a convenient term for the larger undescribed spores.

Other types of spores occur abundantly in the Devonian rocks. Some of these have been found in organic connection with *Archaeopteris, Rhynia, Horneophyton* and others. Spores of the Devonian period have not received adequate attention, but should be a promising field for study.

From the Upper Paleozoic rocks hundreds of species of isolated and unassigned microfossils have been described, and eventually many more will be distinguished. In the coals of the Carboniferous

and Permian periods the fossils consist mainly of pteridophyte spores and pollen of the more primitive gymosperms. One of the surprising facts in microfossil work is the marked uniformity of spore and pollen types that are encountered in widely separated regions. For example, many of the fossils of equivalent ages described from the coals of Russia, Africa and North America are almost identical.

Witham, 1833, appears to have been the first to have described spores in any coal (93). In his classical work on the "Internal Structure of Fossil Vegetables Found in the Carboniferous and Oolitic Deposits of Great Britain", he notes "traces of organisation". These "traces" were probably megaspores, but the identity of the structures were unknown to him. He was inclined to consider them the vessels of monocotyledons.

The first author to figure isolated fossil megaspores was probably Morris (47); however, in the description he regarded them as sporangia of *Lepidodendron* (*Lycopodites*) *longibracteatus*. Four years later Göppert (24) described and figured other megaspores under the name of *Carpolithes coniformis*.

In the subsequent few years other spore descriptions appeared in conjunction with cone studies. Hooker (32) described specimens of *Lepidostrobus* bearing microspores. Carruthers (12) described *Flemingites* sporangia which in reality are megaspores. Schimper (68) first described a fossil cone bearing *in situ* both mega- and microspores. The memoirs of Williamson (86) furnish numerous early descriptions and illustrations of spores both isolated and within their sporangia.

In 1884 Reinsch (66) published an important two volume work in which he figured over one hundred plates of micro-organisms from the coals of Russia and Saxony. Most of these are of spores or portions of spores. The work represents a careful and painstaking study, but unfortunately Reinsch thought the spores were algae, and the spore appendages, which are frequently complex, he considered to be parasites upon the algal cells. Reinsch's contribution to the microfossil content of coal was discounted because of his erroneous conclusions, but regardless of this incorrect interpretation, his work will remain a classic in plant micropaleontology. From Reinsch's investigations the only genus now recognized is *Triletes*.

Two years later Bennie and Kidston (3) read a paper before

the Royal Physical Society, "On the Occurrence of Spores in the Carboniferous Formation of Scotland". In this paper they described and figured numerous megaspores without applying specific names to them. Bennie and Kidston at that time pointed out the mistaken ideas of Reinsch.

During the next two decades most of the fossil spore and pollen descriptions were given in conjunction with morphological studies of fructifications.

Some twenty years ago, Thiessen and his co-workers began a microscopic study of the eastern American coals with the object of correlating various seams by their organic content (79, 80, 81). The results were positive, but limited by the use of thin section methods. In thin sections of coal only a single view of a spore is obtained, which is usually not enough for the identification of most species. With the same objective, Slater, Evans and Eddy (77) began an examination of the English coals. Nineteen possible types were isolated and figured. All of their work, like Thiessen's was done with thin sections of coal, and consequently was limited in results.

In Germany and in England there appeared in 1931 and 1933 studies that have greatly stimulated the study of pre-Quaternary plant micropaleontology. R. Potonie (56) described a number of Paleozoic spores under the generic name *Sporites* of H. Potonie (53). Ibrahim (33, 56) and Loose (40, 56) in Germany began a comprehensive investigation of the taxonomy and geological range of fossil spores. Raistrick and Simpson (63) in England attempted the correlation of some Northumberland coals by spore content. In England the investigations emphasized the stratigraphic values of microfossils and no attempt was made to construct a binomial system of names that would be acceptable to international taxonomic practice. Much of the British work was confined to the study of the smaller spores, since they are considered as being more widely and uniformly spread by the wind and therefore more useful as horizon markers.

At the present time no general agreement exists concerning the classification of the Carboniferous spores and pollen. Raistrick and Simpson (63) devised a system of six spore groups, each of which is known by a letter of the alphabet, A to F inclusive. The various members of each group are then designated by a subnumber such as

A_1, A_2, A_3. The one good feature about the system is that it is a quick, easy method of treating large numbers of fossils both taxonomically and graphically. The continental workers, on the other hand, have established a binary system for the description of unassigned spores and pollen. The system is based upon the character of the exosporium and is definitely artificial. The genus *Sporites* of H. Potonie (53) was early used for spore descriptions, but this genus does little more than designate that the fossil is a spore. Ibrahim (33) proposed another system, which though more comprehensive is still very artificial. This new system proposed that all fossil spores be grouped according to their manner of dehiscence during germination. Three groups known as "Triletes", "Aletes" and "Monoletes" were established, and into these were placed spores whose dehiscence scar was triradiate (trilete), absent (alete) or single (monolete). Genera based upon spore wall characters were set up within each of the groups. Three spores differing in dehiscence scars but similar in wall ornamentation are treated as belonging to three separate genera; for instance, a "Triletes" spore with punctate walls belongs to the genus *Punctati-sporites,* an "Aletes" spore with punctate walls belongs to the *Punctata-sporites,* and a "Monoletes" spore with punctate walls belongs to the genus *Punctato-sporites.* Species within these genera are described in the usual manner.

In the above classifications no attempt was made to separate the large and small spores into morphological entities such as mega-, micro- or isospores. Several of Ibrahim's genera have genotypes that are megaspores, while most other members of the genera are microspores or isospores. In the construction of a natural classification of fossil spores it is important that all possible morphological characters be utilized. Therefore, since the large spores are usually megaspores and the small spores usually microspores or isospores, a more fundamental and natural classification may be made if spore size be included than if the classification be based on dehiscence scars alone. With an aim to summarizing the present knowledge of Paleozoic spores, and solving the taxonomic confusion that exists concerning these fossils, Schopf, Wilson and Bentall (71) have separated the large and small spores, and considered the validity of each pre-existing genus in accordance with the international rules of nomenclature. The results of this intensive search of the litera-

ture has brought together several hundred species of microfossils that are taxonomically valid. Of this number nearly one hundred are megaspores belonging to the genus *Triletes* Reinsch; Schopf emend (70). The genus *Triletes* is at present divided into four sections, and may be further subdivided when it is better understood. Most studies of the larger spores have been made in Europe; some of the most important are the papers of Zerndt (97, 98, 99, 100, 101, 102). In the United States Bartlett (2) has described the presence of *Triletes* in glacial drift pebbles near Ann Arbor, Michigan, and Schopf (70) has described the occurrence of *Triletes* in the Herrin (No. 6) coal bed in Illinois.

The small spores of the Paleozoic are usually less than 100 microns in size. They can be considered mainly microspores and isospores. Some of the larger forms of these may prove to be megaspores, while others are probably pollen of primitive gymnosperms, but the determination of these morphological truths must wait the further study of cones and other spore-bearing structures. Though affinities of many of the isolated spores of the Carboniferous rocks are known or suspected, there remain scores that are still obscurely associated with the plant kingdom. In the classification proposed by Schopf, Wilson and Bentall (71) an attempt is made to bring together isolated forms that resemble the spores known from fructifications of various natural groups of fossil plants. For example, the thin-walled, transparent, spherical or subspherical spores possessing a triradiate tetrad mark are similar to the spores found in the cones of the Calamitaceae. The resemblance is great enough to suspect that the isolated spores are from fossil plants of that family; consequently they are placed in the genus *Calamospora*. Likewise, the small isolated spores resembling those found in the cones of lycopodaceous fossil plants are placed in the genus *Lycospora*. Not all spores can be as easily placed as these two examples, and in some cases, the generic names are not appropriate, a condition that cannot be entirely remedied with adherence to the rules of taxonomy.

The stratigraphic value of microfossil studies is now proved by the careful studies in Europe (63, 64, 44, 49, 41) and America (80, 81, and Bentall (unpub.)). In Britain the correlation of coal seams by spore content has been remarkably successful. Some difficulty, however, has been experienced in attempts to correlate the thicker seams. In Russia spore-complexes have been used to establish the

geological provinces of the Moscow Basin, Kizel Basin, the Pechora River, the Voronezh region, the Berchogur deposit, Spitzbergen (town of Pyramida), Northumberland, the Karaganda Basin, Donetz Basin, and several others of Carboniferous age. According to Naumova (49), in Russia "the geographic complexes of spores and pollen distinguished make it possible to establish the largest stratigraphical subdivisions of the coal-bearing beds and to correlate individual horizons within the beds".

Though early stratigraphic use was made of spores in this country, the method has not been as widely pursued as in Europe. As pointed out above, Thiessen's work on our eastern coals was done with thin sections, and consequently the full value of the fossils as horizon markers could not be attained. Since then, Bentall, with maceration methods, has demonstrated the stratigraphic value of microfossil studies in the coals of Tennessee. Other work now in progress in Illinois, Iowa, Pennsylvania and West Virginia further bears out this fact. Recently, several descriptive studies of the smaller Carboniferous (Pennsylvanian) spores have been made in the United States (5, 38, 70, 71, 88, 89). These indicate the growing interest in the establishment of a valid classification of spores and pollen in order that further stratigraphic use may be made possible.

MICROFOSSILS OF MESOZOIC AGE

Plant microfossils of the Mesozoic rocks have been neglected. The reasons are probably to be found in the comparatively small commercial value of Mesozoic coals in the past, and the apparent lack of coal-balls within these rocks. Much of the paleobotanical work of the Mesozoic has been done with impression material, and it is only since the refinement of microtechnique methods that any extensive microscopic study has been made. Coals of Cretaceous age are very abundant, but those of Jurassic and Triassic age are scarce. Unlike the Paleozoic microfossils, those of the Mesozoic are chiefly pollen, with spores in the minority. In the lower Mesozoic rocks the fossil pollen appears to be entirely of gymnosperms, but from early Jurassic time, pollen of angiosperms becomes increasingly abundant.

The Triassic spores and pollen of the Deep River coal field of North Carolina have been investigated by Dr. Willard Berry, but the study is at present unpublished. The microfossils seem to con-

sist mainly of spores, some of which resemble Paleozoic forms, but some are definitely like conifer pollen.

Another deposit that is of great interest occurs in the Petrified Forest of Arizona. Daugherty (16), while examining the microfossils, also discovered conifer-like pollen as well as several types of spores. One of the latter he refers to a new genus, *Equisetites,* because it closely resembles modern *Equisetum* spores.

Studies of Triassic rocks in the Salt Range of India have revealed spores that have been referred to the genus *Triletes,* and others tentatively to *Sporites* (51).

Jurassic and Cretaceous rocks contain some of the most interesting problems for the micropaleontologist, since during these periods the vegetation of the world underwent profound changes. The Jurassic period was a time of diminishing gymnosperm importance and the beginning of angiosperm floras, which by Cretaceous time dominated the Mesozoic landscapes. Simpson (76) has described an interesting collection of pollen from the Jurassic coal of Scotland. The seam has a rich pollen content made up principally of conifers belonging to the Abietineae. In addition, well preserved pollen of water-lily and possibly *Magnolia* are present. From other English sources spores of several Jurassic ferns, and megaspores belonging to *Triletes,* have been described (34, 48, 82). From the Lias of Greenland Harris (31) has secured many fern spores and conifer pollen associated with vegetative fossil remains. Likewise, the sedimentary rocks of Russia and India (49, 65) contain many microfossils, some of which may be considered pollen of conifers. The Jurassic rocks of North America have not been carefully explored for spores and pollen largely because there is a scarcity of nonmarine deposits.

The abundance of Cretaceous coals and suitable shale formations in North America make these rocks a fertile field for investigation. The Cretaceous coals and shales extend from the Gulf region northward through the interior of the continent to the Arctic. Other suitable Cretaceous deposits in North America are abundant but are not so continuous and widespread. The north-south distribution of Cretaceous deposits should be of great interest to the geographic studies of late Mesozoic floras. At present, only one American Cretaceous spore study has been published (46). In this paper Miner has described several spores which resemble those found

associated with the Mesozoic ferns, *Gleichenites, Gleichenopteris, Laccopteris* and others. Cretaceous studies in Greenland (1, 45) have revealed numerous megaspore fossils that are now referred to the genus *Triletes*. Russian and South African deposits of Upper Cretaceous age contain considerable fossil pollen in addition to spores. Kirchheimer (37) has described a South African deposit containing pollen belonging to the modern families of Pinaceae, Betulaceae and Myricaceae.

MICROFOSSILS OF CENOZOIC AGE

The abundance of fossil spores and pollen in the Cenozoic rocks is remarkable. Early Tertiary deposits reveal the growing dominance of angiosperm pollen. Spores resemble those of the modern ferns *Thelypteris, Dryopteris* and *Lygodium*. Several lycopod and *Equisetum* types are also found, as well as a large quantity of fungal spores. The pollen is definitely modern in appearence, and both arborescent and herbaceous genera are represented. The gymnosperm representatives consist of pine, spruce, fir, cypress, hemlock, *Cycas, Zamia, Cedrus, Glyptostrobus, Cunninghamia, Sequoia, Ephedra* and others. The angiosperm trees include birch, oak, hickory, maple, basswood, elm, walnut, tulip, sour gum, willow and *Engelhardtia*. The herbaceous members present many difficult problems of identification but microfossils of the genera *Potamogeton, Typha, Smilax, Vitis, Myriophyllum, Rumex,* and of the families Araceae, Cyperaceae, Gramineae, Liliaceae, Caryophyllaceae, Ericaceae and Compositae have been recognized.

Some of the earliest investigations of Tertiary plant microfossils were made in Germany (6, 36, 54, 55, 57, 96). In those and subsequent studies several hundred species of fossil spores and pollen have been described and figured. The work indicates the richness of European deposits, and the great care taken to present the material makes the contributions valuable and outstanding. With the discovery of many problematical pollen fossils the question of classification has naturally appeared. This problem was solved temporarily by establishing the genus *Pollenites* (54) and treating the various fossil pollen as species within that genus. Such a lumping of forms is simply for description and does not promote the development of a natural system of classification. Potonie and other German workers have modified the procedure in cases where the phy-

logenetic affinity of the fossil is established. This is done by combining a generic name with an hyphenated suffix, as for example in *Tiliae-pollenites and Ulmi-pollenites.* Such a procedure is burdensome and unnecessary, for the most recognized Tertiary forms probably belong to existing genera or families. Others have preferred to place certain forms in modern genera (*e.g.,* 75, 95). Dr. Wodehouse (95) has made the suggestion that the term *Pollenites* be contracted to '*-pites*' and used as a suffix in the manner described as follows: "Thus a grain which is certainly that of *Pinus,* and which resembles most closely that of the living *Pinus Strobus,* is called *Pinus strobipites.* And the pollen of *Ephedra,* which is the first record of the genus in the Eocene in America, may be called *Ephedra eocenipites.* But, if the genus is not accurately or certainly known, the termination '*-pites*' is applied to the generic designation of the fossil grain instead of to its specific designation. Thus a grain which is known to belong to the Ericaceae, but the genus of which cannot be determined, is called *Ericipites longisulcatus,* for example, the specific name having reference to the length of its furrows. And a grain that matches the living species of *Smilax* but also matches equally well those of some other genera is called *Smilacipites molloides,* for example, its specific designation referring to its further resemblance to the grains of the living *Smilax mollis.* If at some later date any of those genera which bear the termination '*-pites*' should become more closely defined or proved to be accurately determined, the termination may then be transferred to the specific names. The advantages of this system are that complete freedom is allowed in the use of descriptive adjectives as specific names without the introduction of trinomials, some idea is conveyed of the closeness or reliability of the determination, and always is shown the fact that the determination is based on a fossil pollen grain". The suggestions have real merit, but the transfer of the suffix '*-pites*' may easily inject taxonomic errors into the literature.

Oil shales long have been known to contain much organic material and those in Colorado known as the Green River shales (Eocene) are unusual in the amount of spore and pollen fossils they contain. Bradley (8) has described the geology of the deposit and Wodehouse (94, 95) the pollen. Forty-three species in thirty-four genera have been described, only one of which cannot be assigned to a living genus or family. Twenty-one genera are recorded for the first time

in the Green River flora, which is testimony of the manner in which microfossil studies may supplement the macrofossil record. Wodehouse also states that there still remain possibly twice as many undescribed forms in the material that he studied. Wilson and Webster (unpublished) have studied Wyoming coals, also of Green River age, and have distinguished nearly two hundred species of spores and pollen. Many of the forms are identical with those described by Wodehouse. In each case the fossils indicate that the Eocene forests of the Middle Rocky Mountain region were to a large degree similar in generic composition to those now existing in the Northern and Middle Atlantic states. Several interesting additional forms, such as *Ephedra, Glyptostrobus, Cunninghamia* and *Engelhardtia,* have been found in these microfossil floras.

From the Tertiary lignites (Eocene) of west Scotland, Simpson (75) has described pollen fossils belonging to twenty genera. These show a marked resemblance to the East Asiatic flora and certain forms now confined to Africa and America.

The younger Tertiary deposits of North America have not been investigated for plant microfossils, but numerous published studies in Germany (57, 96) indicate that the flora of Europe had become very modern in aspect.

With the coming of the continental ice sheets and the uplift of certain regions of the earth, the Tertiary period came to a close and the Quarternary period began. The microfossil literature for the Quaternary period has been reviewed in this journal (9, 72). Since the publication of Cain's review, the work by investigators in North America may be briefly summarized as (*a*) extending the geographic range of peat studies, (*b*) investigating the interglacial peat deposits, (*c*) correlating of pollen spectra by stratigraphic methods, (*d*) tracing the postglacial migration of forest elements, and (*e*) testing out theories of climatic sequence.

The geographic range of peat studies has been greatly extended and intensified within the United States and Canada. Those workers most active in the field at the present time are Cain, Deevey, Hansen, Knox, Potzger, Sears, Webster, and Wilson. The literature is too extensive to list here, but may be found in the "Pollen Analysis Circular" edited by Dr. Paul B. Sears of Oberlin College.

Buried interglacial silts and peats have revealed much concerning the distribution of our forests during the glacial period. This is

fortunate, for the peats that were present as surface deposits on the older glacial drift plains have largely disappeared as the result of oxidation and erosion. Lane, Voss, and others (39, 84) have described the fossils of the Pleistocene peat deposits of the middle west, Hansen (25) has worked on the west coast, and Knox (67) in the east. The most complete series of interglacial peats that have been described are from the middle west, which is to be expected, since there the drifts are better represented. All of the interglacial deposits except the earliest, the Aftonian, have to date indicated that the Pleistocene forests of northern United States consisted mainly of spruce, fir and pine (39, 90). At the start and end of the Aftonian stage the forests of the middle west also consisted of spruce, fir and pine, but during the middle of the period the forests of Iowa, southern Minnesota and probably Wisconsin contained considerable oak, hickory, maple, basswood and elm.

Correlation studies, though still not abundant, indicate that much can and will be done in the near future. When peat workers attain greater knowledge of Pleistocene geology we can expect more studies of this type. Voss (83) has made a comparative study of bogs on Cary (3rd Wisconsin) and Tazewell (2nd Wisconsin) glacial drift in Illinois. He found that the forest succession on these two closely associated glacial drifts was much alike. Numerous recent papers by Dr. Potzger and his students (58, 60, 61) indicate by the constancy of the pollen curves the stratigraphic use that can be made of pollen spectra within a region. Wilson (87) applied stratigraphic methods to peats associated with various glacial lake stages in northwestern Wisconsin. By these methods the forests were shown to have developed according to the usual order of succession (spruce, pine, deciduous) upon each succeeding plain as the lake levels became progressively lower.

The geographic density of peat studies in North America is now sufficient to allow summaries of various types to be drawn. One of these is the postglacial migration of the forest elements. From 111 peat deposits in the eastern and middlewestern states and northeastern Canada, Sears (74) has traced the postglacial migration of *Quercus, Tsuga, Carya, Fagus and Tilia.* In the northeastern part of the area, the above genera appeared in peat profiles in the following order: *Quercus, Tsuga, Fagus, Carya* and *Tilia.* In the southwestern part, *Quercus, Carya, Tilia, Tsuga* and *Fagus* is the order of

appearance. Important centers of dispersal appear to have existed in the southeastern part of the area and in Wisconsin, possibly in the Driftless Area. Sears correlates the migration of the trees with climatic fluctuations, and points out that *Carya* pollen occurs as fossils in peat, north of the present range of that genus. This latter distribution has received additional discussion (91, 92, 10). The former authors contend that though fossil *Carya* pollen is small in its relative abundance when it occurs considerably north of its present range, it is present only in the higher middle levels of the peat deposits and is consistently absent from the surface layers; therefore, the genus should be considered as having had a more northern range. Cain contends that the fossils probably represent pollen that was wind-borne from a more southern location.

Forest sequence studies (73) from pollen spectra in Illinois, Indiana, Michigan and Ohio suggest that there were two general periods of retrogression in that region. They are assumed to have been due to climatic causes which produced a less favorable water balance. For northern Wisconsin, Minnesota and western Ontario there is good evidence for only one forest retrogression (58, 60, 91). This difference in pollen evidence may prove later to be related to glacial history, since the bogs described by Dr. Sears are located on Cary drift (3rd Wisconsin), while the more northern deposits are developed on the Mankato drifts (4th and 5th Wisconsin).

Climatic inference has long been an important part of Quaternary micropaleontology. At present there are several theories of postglacial climatic history. Smith (78) has attempted the correlation of 148 eastern North American pollen profiles with a view to their climatic inference. He has come to the conclusion that the major European climatic periods (preboreal, boreal, Atlantic, subboreal, subatlantic) are evident in the profiles from the entire eastern North American region. He further states: "The above major climatic periods are indicated in the profiles from the entire region. The profiles from the region are also consistent in showing minor variations of climate within these major periods. These minor variations, occurring almost rhythmically, show that while the European divisions of postglacial climate outlined above apply in a general way to the eastern North American profiles, postglacial climate in eastern North America is perhaps better characterized as a series of climatic pulsations of more or less humid periods".

Sears (74) has suggested the following scheme of climatic changes for eastern North America:

V. The present—probably cooler and with more available moisture than in IV.

IV. A warm dry period—maximum of oaks and hickories, minimum of beech.

III. A more humid, also dry, period—maximum of beech, and, in places, of hemlock.

II. A dry, probably warmer, period—maximum of pine, often with oak.

I. A moist cool period—maximum of fir and spruce.

In the Lake Superior region of northern Wisconsin, Minnesota and Ontario, Wilson and Webster find a sequence of forest changes that they tentatively consider as supporting von Post's view (52). This hypothesis postulates a tri-partite division of postglacial climate: first, a period of increasing warmth; second, a period of maximum warmth; and third, a period of decreasing warmth. The pollen spectra of the Lake Superior region as interpreted by Wilson and Webster agree with periods V and IV of Sears. Period III may later prove to be comparable to the lowest levels of the pollen spectra in the Lake Superior region. Work by Wilson (unpublished) in south central Ohio agrees with Sears' five-fold division of postglacial climate. In southern Ohio the peat pollen record began with the recession of the Tazewell (2nd Wisconsin) ice, while in the Lake Superior region the record began only after the melting of the Later Mankato (5th Wisconsin) ice. Just how much time elapsed between these substages of the Wisconsin glaciers is not known, but the difference in pollen spectra on the drifts would suggest that the time interval was not short. Potzger (58) does not favor the assumption that the present is a period of decreasing warmth. He states: "so many concrete evidences have been published in recent years which show definitely the opposite of such a conception that one reads such postulations with suprise". It might be stated that for each citation given by Dr. Potzger as against the idea, there are others, some of which are by the same men but more recent, that favor the theory of decreasing climatic warmth (13, 42).

For the Spokane, Washington and northern Idaho regions, Hansen (26) has postulated a fivefold postglacial sequence of climate. He states that the initial period was cool and medium dry; second,

there was increasing warmth and dryness; third, further drying and warming; fourth, increasing coolness and moisture; and fifth, coolness and medium dryness. For western Oregon and northwestern California, he indicates (28, 29) that there appears to be some agreement with the von Post scheme of climatic succession. For the insular region of Washington he finds (30) that there is little evidence for climatic trends, and that it is probable that the ocean has served to maintain an equable climate during postglacial time.

SUMMARY

1. Spores and pollen have been abundantly preserved as microfossils in certain sedimentary rocks from Paleozoic time to the present.

2. Objectives in pursuing the study of plant microfossils are: (*a*) to establish the biological affinities of isolated fossil spores and pollen, and to determine the phylogenetic relationships; (*b*) to use spores and pollen as indices in the stratigraphic study of rocks; and (*c*) to aid in the understanding of paleogeographic and paleoecologic problems.

3. The methods of microfossil study are briefly discussed under the various phases of collecting, preparation by isolation, thin sectioning, nitrocellulose peels, maceration, and dispersal, staining, mounting, photographs and classification.

4. Plant microfossils of the Paleozoic rocks consist mainly of pteridophyte spores and the pollen of primitive gymnosperms. Several hundred species have been described. Attempts to use microfossils as horizon markers have been successful.

5. The early Mesozoic plant microfossils are predominantly pteridophyte spores and gymnosperm pollen, but those of the upper Mesozoic rocks contain, in addition, quantities of angiosperm pollen.

6. Cenozoic microfossils consist largely of angiosperm pollen, many of which belong to modern genera.

7. The recent Quaternary microfossil studies in North America indicate that pollen spectra may be used for stratigraphic correlation of interglacial and postglacial sediments. The migration routes of certain forest elements have been traced. The theories of climatic change in postglacial time based on microfossil studies show some refinement, but are as yet tentative.

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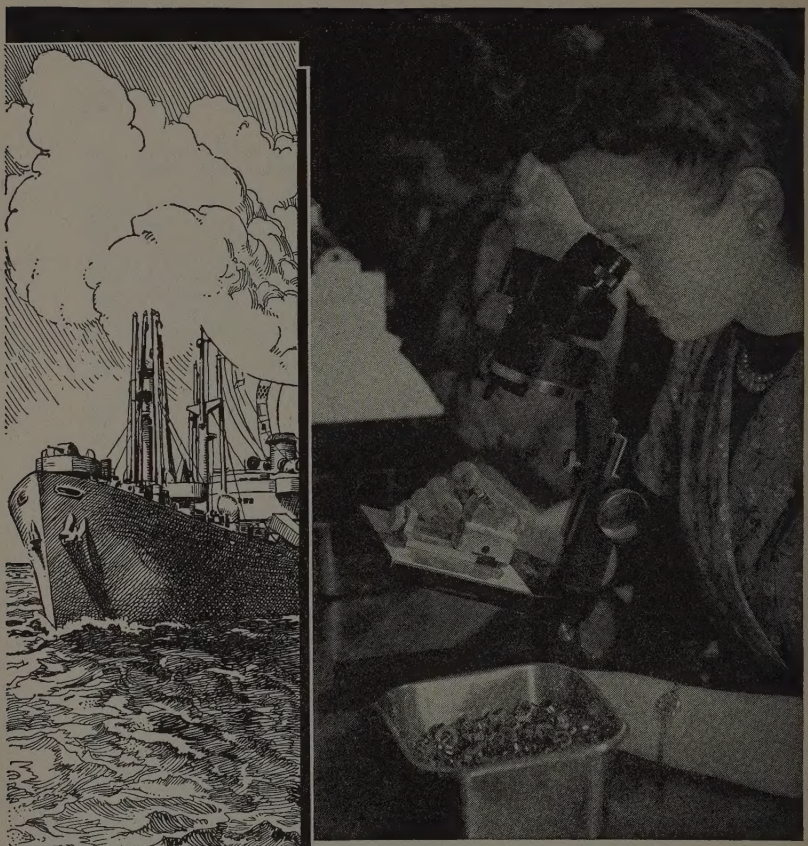

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Proposed Future Contents of
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Articles received and awaiting publication

Antibiotic Substances Derived from Micro-organisms	J. C. HOOGERHEIDE *Corsan Research Laboratories*
Plant Fats in Relation to Environment and Evolution	JAMES B. MCNAIR

Articles arranged for most recently

Mineral Nutrition of Plants as Affecting Mineral Nutrition of Animals, Including Man	L. A. MAYNARD and K. BEESON *Cornell University*
Rubber Formation in Plants	JAMES BONNER and D. A. GOLDSTON *California Institute of Technology*

Articles in course of preparation

The Cytology of Fertilization in Angiosperms	L. E. ANDERSON *Duke University*
Development of the Madro-Tertiary Flora	D. I. AXELROD *University of California*
Relation of Wood Anatomy to Taxonomy	I. W. BAILEY *Harvard University*
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The Genetics of Pollen Tube Growth	J. T. BUCHHOLZ *University of Illinois*
Sexuality and Genetics of Algae	H. BOLD and W. G. WHALEY *Columbia University*
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